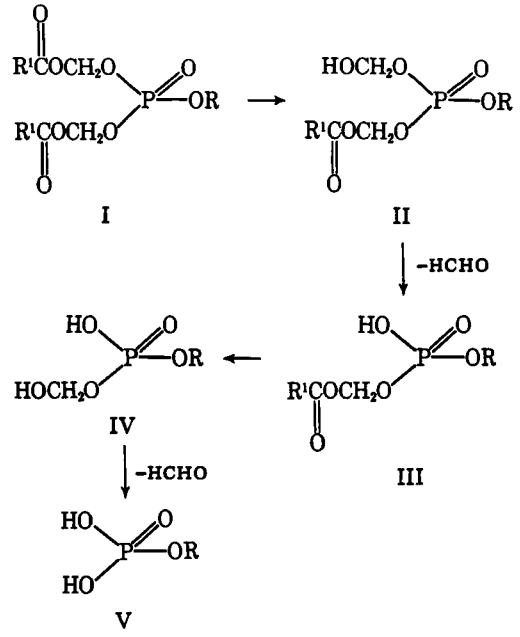
Biologically Reversible Phosphate-Protective Groups

Keyphrases □ Phosphate-protective groups—biologically reversible, acyloxymethyl groups □ Acyloxymethyl groups—biologically reversible phosphate-protective groups □ Chemotherapeutic agents—biologically reversible phosphate-protective groups, acyloxymethyl groups, as a means of traversing biological membranes

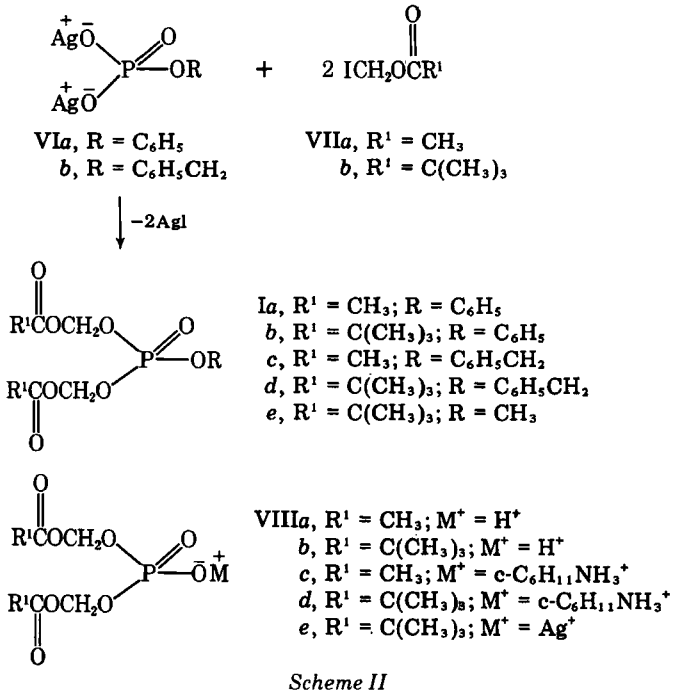
To the Editor:

The inability of nucleotides and other ionic organophosphate esters to traverse biological membranes (1, 2) constitutes a major impediment to the use of such compounds as chemotherapeutic agents. In an attempt to overcome this limitation, we have investigated the potential of acyloxymethyl groups as biologically reversible phosphate-protective groups. Conceivably, neutral acyloxymethyl phosphotriesters[1] could penetrate cell membranes by passive diffusion and revert, intracellularly, to the parent ionic phosphate after cleavage of the acyloxy group by carboxylate esterase and elimination of formaldehyde (Scheme I). To explore synthetic routes to bis(acyloxymethyl) phosphotriesters and to investigate the chemical properties of this class of compounds, we chose phenyl phosphate as a model organophosphate monoester.



Scheme I

Reaction of disilver phenyl phosphate[2] (VI*a*) with a 2.5 mole excess of iodomethyl acetate (VII*a*) (3) in anhydrous benzene at room temperature for 5 hr gave bis(acetoxymethyl) phenyl phosphate[3,4] (I*a*) in 53% yield. Bis(pivaloyloxymethyl) phenyl phosphate[3] (I*b*) was prepared similarly from (VI*a*) and iodomethyl pivaloate (VII*b*) (3) in 54% yield (Scheme II).



Scheme II

Compound I*a* was stable in neutral aprotic solvents (*e.g.*, benzene, diethyl ether, ethyl acetate); however, in protonic solvents (*e.g.*, ethanol, water, 0.05 *M* potassium phosphate buffer, pH 7.4) it was converted[5] slowly ($t_{1/2}$ > 4 hr) to mono(acetoxymethyl) phenyl phosphate (III, R = C_6H_5, R^1 = CH_3). This product is presumably formed by solvolysis of I*a* to the hydroxymethyl intermediate[6] (II, R = C_6H_5, R^1 = CH_3), followed by spontaneous loss of formaldehyde. When incubated at 37° in 0.05 *M* potassium phosphate buffer (pH 7.4) with hog liver carboxylate esterase[7] (E.C. No. 3.1.1.1) (1.6 mg of protein/ml) or mouse plasma (50% by volume), I*a* (2 m*M*) was rapidly degraded[8] ($t_{1/2}$ < 15 min), first to the mono(acetoxymethyl) analogue

[1] To the best of the authors' knowledge, such compounds have not previously been reported.

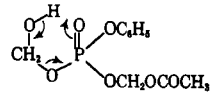
[2] Obtained from the corresponding disodium salt by reaction with silver nitrate in water.

[3] Obtained as a viscous oil.

[4] All compounds gave satisfactory elemental analyses and exhibited spectral characteristics (NMR, MS, IR) consistent with the assigned structures.

[5] The solutions were analyzed by high-performance liquid chromatography (HPLC) (Waters model ALC 204). The disappearance of I was monitored by reverse-phase chromatography on a column of μBondapak-C_{18} (30 cm × 4-mm i.d., 10 μm; Waters Assoc., Milford, Mass.) using solutions of 0.01 *M* potassium phosphate buffer (pH 7.0)–methanol (various proportions, typically 25–50% alcohol) as mobile phase. The formation of III and V was monitored by ion-pair chromatography on μBondapak-C_{18} using the same buffer system as described for I except that tetrabutylammonium hydroxide was added to a concentration of 2×10^{-3} *M*, or by anion-exchange chromatography on a column of Partisil SAX (25 cm × 4.6-mm i.d., 10 μm; Whatman) using a linear gradient of 0.01–0.1 *M* potassium phosphate buffer (pH 6.5) as eluent. The flow rates ranged from 1.0 to 2.0 ml/min. The column effluents were monitored at 254 nm with a Schoeffel model 450 UV detector, and the concentrations of I, III, and V were determined by comparison of the peak areas with those of reference standards.

[6] A labile intermediate was detected in some solutions by HPLC but was not characterized. The hydroxymethyl compound II (R = C_6H_5, R^1 = CH_3) is expected to facilely dissociate, probably by a six-membered cyclic transition state, to give mono(acetoxymethyl) phenyl phosphate and formaldehyde:



[7] Obtained from Sigma Chemical Co., St. Louis, Mo.

[8] At appropriate intervals, aliquots (100 μl) of the incubation mixtures were diluted with 3 volumes of methanol than agitated for 1 min on a Vortex shaker. The precipitated protein was separated by centrifugation at 10,000×*g* for 5 min, and the supernatants were analyzed by HPLC as described in footnote 5.

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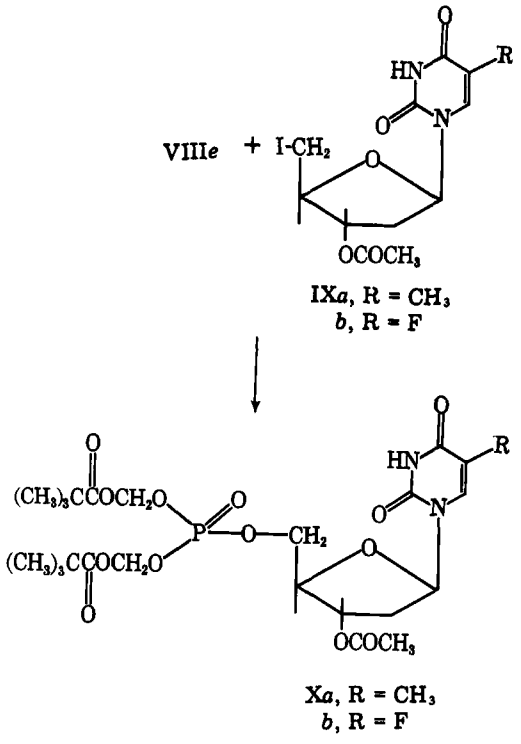
(III, R = C_6H_5, R^1 = CH_3) and then to phenyl phosphate (V, R = C_6H_5).

The bis(pivaloyloxymethyl) phosphotriester[3] (I*b*), by comparison, was much more resistant to both chemical and enzymatic hydrolysis. Thus, it was stable in protonic solvents and had a half-life ≃5 hr when incubated with mouse plasma under conditions identical to that described for I*a*. Clearly, the nature of the acyl substituent has a marked influence on the susceptibility of bis(acyloxymethyl) phosphotriesters to hydrolysis.

The benzyl phosphotriesters, I*c*[3] and I*d*[3], were prepared similarly from disilver benzyl phosphate[2] (VI*b*). Catalytic hydrogenolysis of these compounds over 5% palladium-on-charcoal Pd-C in cyclohexane gave the corresponding monobasic acids, VIII*a* and *b* which were isolated as their cyclohexylammonium salts, VIII*c* and *d*. Silver bis(pivaloyloxymethyl) phosphate (VIII*e*) was prepared from VIII*d* by successive ion-exchange[9]. Reaction of VIII*e* with benzyl bromide or methyl iodide in benzene for 5 hr at room temperature gave bis(pivaloyloxymethyl) benzyl phosphate[3] (I*d*) and bis(pivaloyloxymethyl) methyl phosphate[3] (I*e*), respectively, in nearly quantitative yield. These reactions illustrate the utility of VIII*e* in the synthesis of bis(acyloxymethyl) phosphotriesters.

Reactions of VIII*e* with 5′-deoxy-5′-iodo-3′-*O*-acetylthymidine (IX*a*) (4) in toluene under reflux for 5 hr gave bis(pivaloyloxymethyl)-3′-*O*-acetylthymidine-5′-phosphate (X*a*), 39% yield (Scheme III). Similarly, the reaction



Scheme III

of VIII*e* with 2′,5-dideoxy-5′-iodo-3′-*O*-acetyl-5-fluorouridine[10] (IX*b*) gave X*b* (15% yield). Compound X*b* prevented the growth of Chinese hamster ovary cells in culture (5) at a concentration of 5.0×10^{-6} *M* (5-fluoro-2′-deoxyuridine control, 1.0×10^{-6} *M*).

Further chemical and biological studies of these compounds are in progress.

[9] Prepared on Dowex 50 Na^+ and Dowex 50 Ag^+.

[10] Prepared from 3′-*O*-acetyl-2′-deoxy-5-fluorouridine in 65% yield, according to the general procedure described previously (4).

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Estimation of the Extent of Drug–Excipient Interactions Involving Croscarmellose Sodium

Keyphrases □ Croscarmellose sodium—estimation of the extent of drug–excipient interactions □ Drug–excipient interactions—estimation of the extent involving croscarmellose sodium □ Excipients—estimation of the extent of drug–excipient interactions involving croscarmellose sodium

To the Editor:

In a recent communication (1), a pH-dependent interaction of oxymorphone derivatives with croscarmellose sodium, Type A, NF XV[1] was identified. Any drug–excipient interaction is potentially serious if it has a deleterious influence on the bioavailability of the drug from the dosage form. However, as in this case, the excipient may be responsible for certain dosage form properties which promote or at least ensure reproducible drug delivery.

There is a need to be able to assess the risk which may be involved before advocating or indicting an excipient. The objectives here are to provide a means for determining when an interaction with the disintegrant croscarmellose sodium might be expected and a means for estimating the extent of the interaction when it occurs.

Certain aspects of the interaction were presented in the previous communication (1); although, the general utility of these results is limited. Details were not given con-

[1] Ac-Di-Sol, FMC Corporation, Philadelphia, Pa.

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